Structural Features of Azidopyridinyl Neonicotinoid Probes Conferring High Affinity and Selectivity for Mammalian α4β2 and *Drosophila* Nicotinic Receptors

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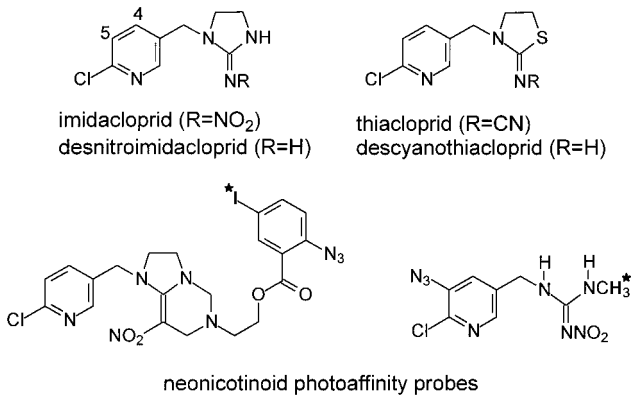
The higher toxicity of neonicotinoid insecticides such as *N*-(6-chloropyridin-3-ylmethyl)-2-nitroiminoimidazolidine (imidacloprid) to insects than mammals is due in large part to target site specificity at the corresponding nicotinic acetylcholine receptors (nAChRs). We propose that neonicotinoids with a protonated *N*-unsubstituted imine or equivalent substituent recognize the anionic subsite of the mammalian α4β2 nAChR whereas the negatively charged (δ^-) tip of the neonicotinoid insecticides interacts with a putative cationic subsite of the insect nAChR. This hypothesis can be tested by using two photoaffinity probes that differ only in the *N*-unsubstituted imine vs negatively charged (δ^-) tip. Synthesis methodology was developed for compounds combining three moieties: pyridin-3-ylmethyl or 6-chloropyridin-3-ylmethyl and their 4- and 5-azido analogues; imidazolidine, 4-imidazoline or 4-thiazoline; and *N*-unsubstituted imine, nitroimine, cyanoimine, or nitromethylene. Structure−activity studies compared displacement of [^{3}H]nicotine binding in mammalian α4β2 nAChR and [^{3}H]imidacloprid binding in *Drosophila* nAChR. Preferred compounds are *N*-(5-azido-6-chloropyridin-3-ylmethyl) with 2-iminothiazoline for α4β2 (K_i = 0.47 nM) and with 2-nitroiminothiazoline or 2-nitromethyleneimidazolidine for *Drosophila* (K_i = 0.72−3.9 nM).

Introduction

Neonicotinoid insecticides are increasingly used in crop protection and animal health care because of their effectiveness and safety.[1] These insecticides exemplified by *N*-(6-chloropyridin-3-ylmethyl)-2-nitroiminoimidazolidine (imidacloprid) (Chart 1) preferentially bind to the insect vs mammalian nicotinic acetylcholine receptors (nAChRs), serving as a basis for selective toxicity.[2] However, the desnitro or descyano metabolites and analogues of nitroimine or cyanoimine neonicotinoids (Chart 1) display high affinity and agonist potency equal to or greater than those of (−)-nicotine at the mammalian nAChR subtypes.[3−6] These findings led to the hypothesis that different molecular features of neonicotinoids are required for selective interaction with mammalian and insect nAChRs.[2]

The nAChR, assembled from five homologous subunits, penetrates the synaptic membrane to form an ionotropic pore. Different subunit combinations lead to receptor subtypes with distinctive pharmacological profiles, since the drug-binding site is localized at the interface region between subunits.[7,8] The α4β2 heteromer is a predominant nAChR subtype expressed in mammalian brain. It represents >90% of high-affinity tritiated agonist binding sites[9,10] and consists of two α4 and three β2 subunits.[11,12] The α4β2 nAChR is the target for several potential therapeutic agents for neuropathic diseases, cognitive enhancement, and analgesia, leading to a current interest in subtype selective compounds.[7,8,13] In contrast, knowledge of the functional architecture and diversity of insect nAChRs is in an embryonic stage.[14,15]

Chart 1. Two Major Neonicotinoid Insecticides (Imidacloprid **2a** and Thiacloprid **4a**), Their Imine Metabolites or Analogues (**1a**,**e**), and Two Neonicotinoid Photoaffinity Radioligands



Photoaffinity labeling of critical amino acid residue(s) in the ligand-binding site is an important approach to understand the functional architecture of the nAChR. Insect nAChRs have been photoaffinity-labeled with radioprobes shown in Chart 1, leading to identification of the insecticide-binding subunit(s); however, these photoaffinity probes are ineffective for the mammalian nAChR.[16−19]

We propose that the protonated *N*-unsubstituted imine or equivalent substituent of neonicotinoid analogues such as desnitroimidacloprid (as with nicotine and epibatidine) recognizes the anionic subsite of the mammalian α4β2 nAChR whereas the negatively

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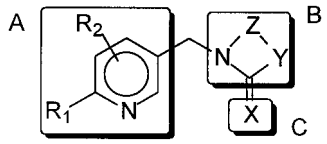


A: 6-chloropyridin-3-yl or pyridin-3-yl and their 4- and 5-azido analogs R_1=Cl or H; R_2=4-N_3, 5-N_3 or H

B: imidazolidine (Y=NH, Z=CH_2CH_2)
imidazoline (Y=NH, Z=CH=CH)
thiazoline (Y=S, Z=CH=CH)

C: imine (X=NH, **1**)
nitroimine (X=NNO_2, **2**)
nitromethylene (X=$CHNO_2$, **3**)
cyanoimine (X=NCN, **4**)

Figure 1. Structural variations of neonicotinoids and their imine analogues examined.

charged (δ^-) tip of the neonicotinoid nitroimine, nitromethylene, or cyanoimine moiety interacts with a putative cationic subsite of the insect nAChR.[2] This hypothesis provides distinct molecular features to distinguish mammalian from insect receptors and can be investigated by using two photoaffinity probes that differ only in the *N*-unsubstituted imine vs negatively charged (δ^-) tip. The strategy for probe selection involved optimizing the combination of three moieties: (i) *N*-(pyridin-3-ylmethyl) or *N*-(6-chloropyridin-3-ylmethyl) and their 4- and 5-azido analogues; (ii) imidazolidine, 4-imidazoline, or 4-thiazoline; and (iii) *N*-unsubstituted imine, nitroimine, nitromethylene, or cyanoimine (Figure 1). In this paper, we report the development of synthesis methodology and structure−activity relationships (SARs) of these candidate photoaffinity probes as key steps in determining structural differences of the binding subsites in the mammalian and insect nAChRs.

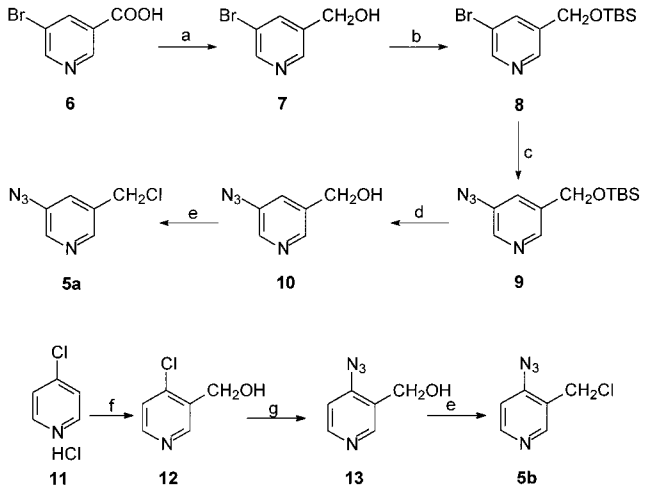
Results and Discussion

Structural Modifications. General. Compounds **1**−**4** can be synthesized by coupling the appropriate pyridin-3-ylmethyl chloride analogues with the heterocyclic moiety. The first step for the azido analogues is therefore to prepare the azidopyridin-3-ylmethyl chlorides (**5**).

Azidopyridin-3-ylmethyl Chlorides (5a,b) (Scheme 1). 5-Azidopyridin-3-ylmethyl chloride (**5a**) was prepared from 5-bromonicotinic acid (**6**). Reaction of **6** with ethyl chloroformate in the presence of *N*-methylmorpholine (NMM) followed by reduction with $NaBH_4$ gave alcohol **7**.[20] Attempts to replace bromo with azido by direct reaction with NaN_3 in dimethylformamide (DMF) at 100 °C were not successful. Accordingly, compound **7** was protected as **8** with *tert*-butyldimethylsilyl chloride (TBSCl). The lithium−bromide exchange reaction with *t*-BuLi followed by treatment with trisyl azide introduced an azido group at the 5-position to give compound **9**, which was deprotected with tetrabutylammonium fluoride (TBAF) to **10** quantitatively. Alcohol **10** was converted to chloride **5a** by dichlorotriphenylphosphorane (PPh_3Cl_2) with NMM under mild conditions.[21]

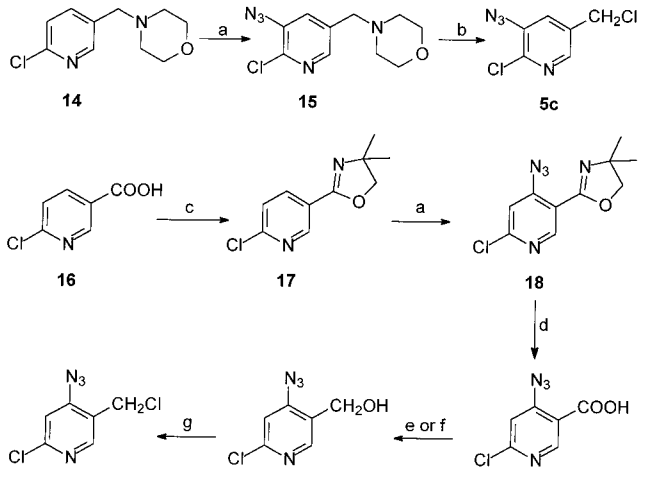
Synthesis of 4-azidopyridin-3-ylmethyl chloride (**5b**) started from 4-chloropyridine (**11**) as the hydrochloride. Lithiation of **11** as the free base with LDA followed by

Scheme 1. Synthesis of Azidopyridin-3-ylmethyl Chlorides **5a**,**b**[a]



[a] Reagents and conditions: (a) (i) $ClCO_2Et$, NMM, THF, −10 °C, 10 min; (ii) $NaBH_4$, MeOH, −78 to 0 °C, 80 min. (b) TBSCl, imidazole, DMAP, DMF, room temperature, overnight. (c) (i) *t*-BuLi, THF, −100 °C, 30 min; (ii) trisyl azide, THF, −100 °C to room temperature. (d) TBAF, THF, room temperature, overnight. (e) PPh_3Cl_2, NMM, CH_3CN, 0 °C, 50 min. (f) (i) $KHCO_3$; (ii) LDA, THF, −78 °C, 1 h; (iii) DMF, −78 °C to room temperature; (iv) $NaBH_4$, H_2O, 0 °C, 1 h. (g) NaN_3, EtOH/H_2O, reflux, 7 h.

Scheme 2. Synthesis of Azido-6-chloropyridin-3-ylmethyl Chlorides **5c**,**d**[a]

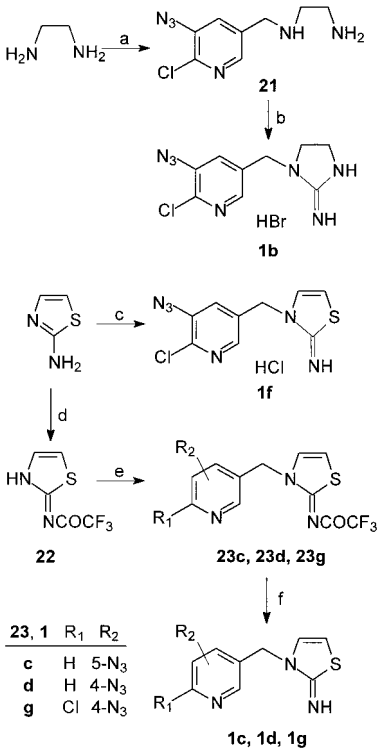


[a] Reagents and conditions: (a) (i) LDA, THF, −78 °C, 5 min; (ii) tosyl azide, THF, −100 to 0 °C. (b) $ClCO_2Et$, reflux, 6 h. (c) (i) $SOCl_2$, reflux, 3 h; (ii) 2-amino-2-methylpropanol, DMAP, CH_2Cl_2, room temperature, 2 h; (iii) $SOCl_2$, room temperature, overnight. (d) (i) Bleach, *n*-Bu_4NHSO_4, EtOAc, room temperature, overnight; (ii) NaOH, MeOH/H_2O, room temperture, overnight. (e) (i) $ClCO_2Et$, NMM, THF, 0 °C, 20 min; (ii) $NaBH_4$, MeOH, 0 °C, 20 min. (f) (i) $SOCl_2$, reflux, 2 h; (ii) $NaBH_4$, H_2O, 0 °C, 15 min. (g) PPh_3Cl_2, NMM, CH_3CN, 0 °C, 40 min.

treatment with DMF provided 4-chloro-3-pyridinecarboxaldehyde as an intermediate, which was reduced to alcohol **12** by $NaBH_4$. The 4-chloro substituent was replaced with an azido group by reacting **12** with NaN_3 to give **13**, which was further transformed to **5b** with PPh_3Cl_2.[21]

Azido-6-chloropyridin-3-ylmethyl Chlorides (5c,d) (Scheme 2). Very different procedures were used to introduce the azido group into the 5- and 4-positions. 5-Azido-6-chloropyridin-3-ylmethyl chloride (**5c**) was

Scheme 3. Synthesis of Imine Metabolites and Analogues of Neonicotinoid Insecticides[a]

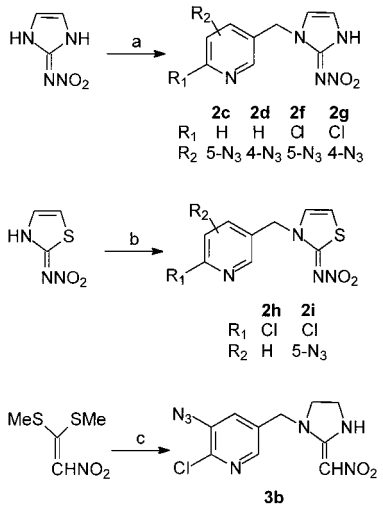


[a] Reagents and conditions: (a) Compound **5c**, NaOH, CH_3CN, 0 °C and then room temperature, 6 h. (b) BrCN, toluene, room temperature, overnight. (c) Compound **5c**, *i*-PrOH, reflux, 10 h. (d) TFAA, pyridine, CH_2Cl_2, room temperature, 30 min. (e) Compound **5a**, **5b**, or **5d**, NaH, DMF, 0 °C to room temperature, overnight. (f) NH_3/MeOH, room temperature, overnight.

synthesized via **14** and **15** as reported; introduction of azido to the 5-position was ortho-directed by the 6-chloro substituent.[18] Synthesis of **5d** required introduction of an azido group at the 4-position, which is also meta to the chloro substituent. Direct lithiation of 6-chloronicotinic acid (**16**) at this position by lithium 2,2,6,6-tetramethylpiperidide has been reported,[22] but this procedure was not successful in the present case to introduce an azido group. Oxazoline was then used as the ortho-directing substituent.[23] Compound **16** was protected as oxazoline **17**[24] and lithiated with LDA followed by treatment with tosyl azide to give 2-(4-azido-6-chloropyridin-3-yl)oxazoline (**18**), with no 5-azido product isolated. Apparently, the oxazoline group has a much stronger ortho-directing effect than chloride so that lithiation occurred only at the 4-position. Subsequent conversion of **18** to 4-azido-6-chloronicotinic acid (**19**) was accomplished using an oxidative cleavage procedure with bleach.[25,26] Other classical methods to break down the oxazoline, i.e., treatment with strong acid,[27] reaction with MeI followed by alkali hydrolysis,[28] or $NaBH_4$ reduction, provided no success. Reduction of carboxylic acid **19** to alcohol **20** was achieved by treatment with either (i) ethyl chloroformate in the presence of NMM and then $NaBH_4$[20] or (ii) $SOCl_2$ followed by $NaBH_4$.[29] Compound **20** was converted to **5d** by PPh_3Cl_2.[21]

Synthesis of Imines (1b−d,f,g) (Scheme 3). Imine **1b** was synthesized as a salt by the cyclization of cyanogen bromide and diamine **21**[29] that was obtained by reaction of **5c** with a large excess of ethylenediamine.

Scheme 4. Synthesis of Neonicotinoid Insecticides with Nitroimine and Nitromethylene Moieties[a]



[a] Reagents and conditions: (a) Compound **5a**−**d**, NaH, DMF, 0 °C to room temperature, 4−12 h. (b) Compound **5c** or 6-chloropyridin-3-ylmethyl chloride, K_2CO_3, DMF, 60 °C, overnight. (c) Compound **21**, *p*-toluenesulfonic acid, CH_3CN, room temperature, 16 h.

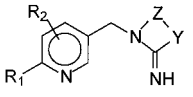
Compound **1f** as a salt was prepared by coupling **5c** with 2-aminothiazole in *i*-PrOH under reflux. However, similar reactions of **5a**,**b** under the same condition did not provide the desired products **1c**,**d**, probably due to the instability of **5a**,**b** under the refluxing condition. In an alternative milder approach, 2-aminothiazole was protected as trifluoroacetamide **22** and reacted with **5a**,**b** or **5d** to produce the corresponding *N*-alkylated compound **23**. Deprotection of **23** with NH_3/MeOH gave **1c**,**d**,**g** in high yields. The alkylation position was identified as the endo-cyclic nitrogen by the chemical shifts of both protons on the thiazoline ring at ∼5.8 and ∼6.5 ppm.[3]

Synthesis of Nitroimines (2c−i) and a Nitromethylene (3b) (Scheme 4). Nitroimines **2c**,**d**,**f**−**i** (as with **2e**[30]) were prepared by the coupling reaction of **5a**−**d** with selected heterocycles in the presence of either NaH or K_2CO_3 with nonoptimized yields of 20−80%. This coupling reaction was not suitable to prepare nitromethylene **3b**, which was instead synthesized by reaction of **21** with 1,1-bis(methylthio)-2-nitroethylene in the presence of a catalytic amount of *p*-toluenesulfonic acid. Nitromethylene **3b** as obtained was identified as the *E*-isomer (the same as its nonazido analogue **3a**[31]) based on a nuclear Overhauser enhancement spectroscopy (NOESY) experiment.

SARs. General. The binding affinities of azidopyridinyl neonicotinoid ligands were determined with $\alpha4\beta2$ and *Drosophila* nAChRs using [^{3}H]nicotine and [^{3}H]IMI, respectively. The corresponding nonazido parent compounds were used for comparison. The overall series has a pyridin-3-ylmethyl moiety with various combinations of azido and chloro substituents coupled to a heterocyclic moiety containing an *N*-unsubstituted imine or a negatively charged (δ^-) tip.

Selectivity. Seven *N*-unsubstituted imine analogues (**1a**−**g**) and (−)-nicotine and (±)-epibatidine are at least 29-fold more potent with $\alpha4\beta2$ than *Drosophila* nAChRs (Table 1). In contrast, nine nitroimines (**2a**−**i**), two nitromethylenes (**3a**,**b**), and two cyanoimines (**4a**,**b**) are

Table 1. Selectivity and Potency of *N*-Unsubstituted Imines (**1**) for Mammalian α4β2 and *Drosophila* Nicotinic Receptors

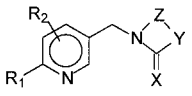


Imidazolidine: Y = NH, Z = CH_2CH_2 Thiazoline: Y = S, Z = CH=CH

compound				$K_i \pm$ SD (nM, $n = 3$)		
	designation	R_1	R_2	α4β2[a]	*Drosophila*[b]	selectivity[c]
imidazolidines	**1a**	Cl	H	2.2 ± 0.3	800 ± 35	364
	1b	Cl	5-N_3	2.6 ± 0.5	5700 ± 260	2192
thiazolines	**1c**	H	5-N_3	8.3 ± 0.8	7200 ± 1400	867
	1d	H	4-N_3	820 ± 90	30 000 ± 2600	36
	1e[d]	Cl	H	0.39 ± 0.09	100 ± 9	256
	1f[d]	Cl	5-N_3	0.47 ± 0.03	750 ± 46	1596
	1g	Cl	4-N_3	110 ± 19	3200 ± 800	29
standards						
	(−)-nicotine			2.0 ± 0.5	2000 ± 100	1000
	(±)-epibatidine			0.010 ± 0.003	220 ± 15	22000

[a] [^{3}H]Nicotine binding to α4β2 nAChR immunoisolated from M10 cells. [b] [^{3}H]IMI binding to *Drosophila* head membranes. [c] Defined as K_i (*Drosophila*)/K_i (α4β2). [d] K_i values of 2000 ± 400 and 3000 ± 650 nM were obtained for compounds **1e**,**f**, respectively, against α7 receptor subtype immunoisolated by monoclonal antibody 306 from human neuroblastoma SH-SY5Y cells and assayed with 1 nM [^{125}I]α-bungarotoxin binding (for methodology, see refs 2 and 4).

Table 2. Selectivity and Potency of Nitroimines (**2**), Nitromethylenes (**3**), and Cyanoimines (**4**) for *Drosophila* and Mammalian α4β2 Nicotinic Receptors



Imidazolidine: Y = NH, Z = CH_2CH_2 Imidazoline: Y = NH, Z = CH=CH Thiazoline: Y = S, Z = CH=CH

compound				$K_i \pm$ SD (nM, $n = 3$)		
	designation	R_1	R_2	*Drosophila*[a]	α4β2[b]	selectivity[c]
			Nitroimines (**2**, X = NNO_2)			
imidazolidines	**2a**	Cl	H	2.2 ± 0.2	720 ± 30	327
	2b	Cl	5-N_3	24 ± 1	340 ± 47	14
imidazolines	**2c**	H	5-N_3	540 ± 35	5400 ± 1320	10
	2d	H	4-N_3	3600 ± 860	95 800 ± 25 000	27
	2e	Cl	H	0.85 ± 0.35	470 ± 92	553
	2f	Cl	5-N_3	13 ± 1.6	300 ± 64	23
	2g	Cl	4-N_3	460 ± 25	16 900 ± 2060	37
thiazolines	**2h**	Cl	H	0.35 ± 0.01	170 ± 11	486
	2i	Cl	5-N_3	3.9 ± 0.4	170 ± 21	44
			Nitromethylenes (**3**, X = $CHNO_2$)			
imidazolidines	**3a**	Cl	H	0.12 ± 0.005	60 ± 6	500
	3b	Cl	5-N_3	0.72 ± 0.10	40 ± 5	56
			Cyanoimines (**4**, X = NCN)			
thiazolines	**4a**[d]	Cl	H	1.4 ± 0.4	120 ± 8	86
	4b[d]	Cl	5-N_3	28 ± 5	120 ± 17	4.3

[a] [^{3}H]IMI binding to *Drosophila* head membranes. [b] [^{3}H]Nicotine binding to α4β2 nAChR immunoisolated from M10 cells. [c] Defined as K_i (α4β2)/K_i (*Drosophila*). [d] Data from ref 19.

4.3−553-fold more potent with *Drosophila* than α4β2 nAChRs (Table 2). This receptor selectivity is in agreement with our earlier hypothesis,[2] i.e., (i) an *N*-unsubstituted imine moiety (which is mostly protonated at physiological pH) specifically interacts with the anionic subsite of the mammalian nAChR and (ii) neonicotinoids with a negatively charged (δ^-) tip due to strong electron-withdrawing substituents selectively recognize a putative cationic subsite in the *Drosophila* receptor.

Potency of *N*-Unsubstituted Imines (Table 1). With the α4β2 receptor, changing the heterocycle from imidazolidine (**1a**,**b**) to the corresponding thiazoline (**1e**,**f**) increases the potency by 6-fold. Introducing the 5-azido substituent does not significantly change the potency as noted by comparing **1a** with **1b** (K_i = 2.2−2.6 nM) and **1e** with **1f** (K_i = 0.39−0.47 nM). Replacing 5-azido with 4-azido (**1d**,**g** vs **1c**,**f**) or deleting the 6-chloro substituent (**1c**,**d** vs **1f**,**g**) leads to a 7−234-fold loss in potency. The high affinity **1f** (K_i = 0.47 nM) combines the 5-azido-6-chloropyridin-3-ylmethyl and 2-iminothiazoline moieties.

Although the *Drosophila* receptor is much less sensitive to these compounds, the SARs generally follow those with the α4β2 nAChR except for the effect of the 5-azido substituent, which greatly decreases potency with *Drosophila* but not with the α4β2 receptor.

Potency of Nitroimines, Nitromethylenes, and Cyanoimines (Table 2). With the *Drosophila* receptor, the heterocyclic moieties can be compared as K_i values for analogous nitroimino compounds. Averaging data for the 6-chloro and 5-azido-6-chloro analogues, the potencies as K_i values decrease in the order of thiazolines (2.1 nM for **2h**,**i**) > imidazolines (6.9 nM for **2e**,**f**) > imidazolidines (13 nM for **2a**,**b**). On the same basis, the nitromethyleneimidazolidines (K_i = 0.42 nM for **3a**,**b**) are more potent than the analogous nitroimines (K_i = 13 nM), and the nitroiminothiazolines (K_i = 2.1 nM) are more potent than the analogous cyanoimines

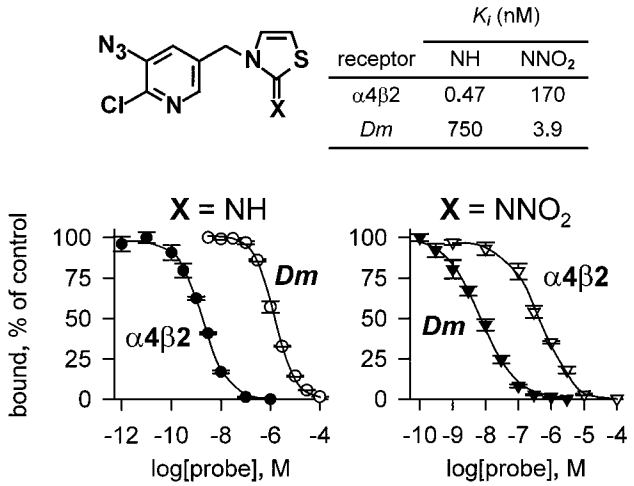


Figure 2. Candidate photoaffinity probes of the imine type (left, X = NH, compound **1f**) and nitroimine type (right, X = NNO_2, compound **2i**), which are potent and selective for the α4*β*2 and *Drosophila* (*Dm*) nAChRs, respectively.

(K_i = 15 nM for **4a**,**b**). Introduction of an azido substituent at the 5-position generally decreases the affinity. Removal of the 6-chloro substituent (**2c**,**d**) or replacing 5-azido with 4-azido (**2d** or **2g**) greatly reduces the potency. Most important is the discovery of two very high potency azidoneonicotinoids (K_i = 0.72−3.9 nM), i.e., nitromethylene analogue **3b** and nitroimine compound **2i**.

The α4*β*2 nicotinic receptor is much less sensitive to this series of compounds than the *Drosophila* receptor, but both receptors follow the same general SARs except that 5-azido greatly decreases potency with *Drosophila* and is without effect or increases potency with α4*β*2 nAChR.

Candidate Photoaffinity Probes (Figure 2). The azidopyridinylmethyl moiety has the expected photoreactivity undergoing complete decomposition in 2 min on irradiation in ethanol at 254 nm (data for **1f** and **2i** not shown). The ideal azidoneonicotionid probe must also have very high affinity at the receptor target. Compound **1f** is the best candidate for the α4*β*2 receptor (K_i = 0.47 nM) and is actually the most potent reported to date. Although compound **3b** has higher affinity (K_i = 0.72 nM) than that of compound **2i** (K_i = 3.9 nM) with the *Drosophila* receptor, **2i** is preferable for radiolabeling due to the ease of the reaction and the greater stability of nitroimines than nitromethylenes.[32,33] The bridging methylene position may be a suitable site for introducing tritium in each case.

Experimental Section

General. Compounds available from or synthesized according to previous studies were **1a**,[3] **1e**,[3] **2a**,[4] **2b**,[18] **3a**,[29] **4a**,[19] **4b**,[19] **5c**,[18] **14**,[18] and **15**.[18] The heterocyclic intermediates were prepared by described procedures, i.e., 2-nitroimino-4-imidazoline,[30] 2-nitroiminoimidazolidine,[34] 2-nitromethyleneimidazolidine,[35] and 2-nitroiminothiazoline.[36] Trisyl azide[37] and tosyl azide[38] were synthesized by reaction of NaN_3 with trisyl chloride or tosyl chloride. (±)-Epibatidine dihydrochloride and (−)-nicotine hydrogen tartrate were obtained from Sigma (St. Louis, MO). Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl. Other anhydrous solvents (DMF, dichloromethane, and acetonitrile) were used directly. Reactions involving the introduction of an azido group were carried out under argon in oven-dried glassware.

All candidate probes and intermediates were identified by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS). The purity of each candidate probe for SAR studies was determined by either elemental analysis or high-performance liquid chromatography (HPLC). ^{1}H and ^{13}C spectra were recorded for solutions in $CDCl_3$ unless indicated otherwise using a Bruker AMX-300 spectrometer at 300 and 75 MHz, respectively. Chemical shifts for ^{1}H and ^{13}C are relative to the solvent chemical shifts. Fast atom bombardment (FAB)-HRMS was performed by the Mass Spectrometry Facility at the University of Notre Dame (Notre Dame, IN). Combustion analyses were performed by Quantitative Technologies, Inc. (Whitehouse, NJ) or the Department of Chemistry at the University of California (Berkeley, CA). Purity determination with HPLC involved a Hewlett-Packard system using a Beckman ultrasphere ODS C18 column (4.6 mm ID × 250 mm). Two solvent systems were employed isocratically or as a gradient to achieve retention times between 5 and 11 min, i.e., varying ratios of 0.05 M NaH_2PO_4 and 60% acetonitrile in 0.05 M NaH_2PO_4 and varying ratios of water and 0.1% trifluoroacetyl (TFA) in acetonitrile. Melting points were measured on a Fisher-Johns melting point apparatus without correction. Column chromatography was performed on silica gel (230−400 mesh), and thin-layer chromatography (TLC) was performed on Merck silica gel plates with a 0.25 mm coating containing fluorescent indicator.

(5-Bromopyridin-3-yl)methanol (7). A solution of **6** (3.0 g, 15 mmol) in THF (75 mL) at −10 °C was treated sequentially with NMM (1.65 mL, 15 mmol) and ethyl chloroformate (1.42 mL, 15 mmol). After 10 min, $NaBH_4$ (1.7 g, 45 mmol) was added followed by MeOH (100 mL) dropwise at −78 °C over 60 min with continued stirring for 20 min at 0 °C. Column chromatography gave **7** as an oil (1.83 g, 66%). ^{1}H NMR: *δ* 8.58 (br s, 1H), 8.49 (br s, 1H), 7.92 (br s, 1H), 4.74 (s, 2H). ^{13}C NMR: *δ* 150.0, 146.4, 138.0, 137.3, 120.9, 61.9.

3-Bromo-5-(*tert*-butyldimethylsilanyloxymethyl)-pyridine (8). A solution of **7** (2.2 g, 11.7 mmol), TBSCl (2.2 g, 14.6 mmol), imidazole (2.0 g, 29.4 mmol), and 4-(dimethylamino)pyridine (DMAP, 50 mg) in DMF (7.0 mL) was stirred at room temperature overnight and then treated with water, followed by three extractions with EtOAc. The organosoluble fractions were chromatographed with EtOAc and hexanes (3:1) to give **8** as a colorless oil (3.2 g, 89%). ^{1}H NMR: *δ* 8.56 (s, 1H), 8.46 (s, 1H), 7.82 (s, 1H), 4.74 (s, 2H), 0.95 (s, 9H), 0.12 (s, 6H). ^{13}C NMR: *δ* 149.4, 145.9, 138.6, 136.4, 120.7, 62.0, 25.8, 18.3, −5.3. FAB-HRMS calcd for $C_{12}H_{21}BrNOSi(MH^+)$, 302.0576; found, 302.0571.

3-Azido-5-(*tert*-butyldimethylsilanyloxymethyl)-pyridine (9). Into a solution of *t*-BuLi (4.0 mL of a 1.5 M solution in pentane, 6.0 mmol) in THF (20 mL) was dropped a solution of **8** (1.5 g, 5.0 mmol) in THF (20 mL) at −100 °C. The solution was stirred for 30 min and treated with trisyl azide (1.86 g, 6.0 mmol) in THF (3.0 mL). The reaction mixture was allowed to rise to room temperature and then poured into saturated NH_4Cl solution. The EtOAc extractable fraction was chromatographed with EtOAc and hexanes (1:4) to give **9** as a pale yellow oil (0.80 g, 61%). ^{1}H NMR: *δ* 8.32 (s, 1H), 8.25 (s, 1H), 7.37 (s, 1H), 4.77 (s, 2H), 0.95 (s, 9H), 0.12 (s, 6H). ^{13}C NMR: *δ* 144.0, 139.9, 138.0, 137.0, 123.6, 62.1, 25.8, 18.4, −5.4. FAB-HRMS calcd for $C_{12}H_{21}N_4OSi(MH^+)$, 265.1485; found, 265.1492.

(5-Azidopyridin-3-yl)methanol (10). A solution of **9** (0.80 g, 3.0 mmol) in THF (6.0 mL) was treated with a solution of TBAF in THF (6 mL of a 1.0 M solution, 6.0 mmol) and stirred at room temperature overnight. The reaction was quenched with saturated NH_4Cl and brine and then extracted with EtOAc. The organic layers were chromatographed with EtOAc to give **10** (0.44 g, 100%). ^{1}H NMR: *δ* 8.32 (br s, 1H), 8.23 (br s, 1H), 7.44 (br s, 1H), 4.75 (s, 2H). ^{13}C NMR: *δ* 144.4, 140.3, 137.5, 137.4, 124.4, 62.0. FAB-HRMS calcd for $C_6H_7N_4O(MH^+)$, 151.0620; found, 151.0623.

5-Azidopyridin-3-ylmethyl Chloride (5a). A solution of **10** (0.45 g, 3.0 mmol) and NMM (0.55 mL, 6.0 mmol) in CH_3CN (15 mL) was cooled to 0 °C. PPh_3Cl_2 (1.6 g, 6.0 mmol)

was added as two portions with an interval of 10 min. After an additional 40 min at 0 °C, MeOH (0.1 mL) and $KHCO_3$ were added at room temperature. Filtration, solvent evaporation, and chromatography with EtOAc and hexanes (1:4) gave **5a** as an oil (0.31 g, 20%). ^{1}H NMR: δ 8.38 (d, 1H, J = 2.0 Hz), 8.31 (d, 1H, J = 2.6 Hz), 7.40 (dd, 1H, J = 2.0, 2.6 Hz), 4.58 (s, 2H). ^{13}C NMR: δ 145.5, 141.2, 137.3, 134.1, 125.8, 42.3. FAB-HRMS calcd for $C_6H_6ClN_4(MH^+)$, 169.0281; found, 169.0298.

(4-Chloropyridin-3-yl)methanol (12). To an aqueous solution of $KHCO_3$ (2.0 g, 20 mmol) was added the hydrochloride of **11** (3.0 g, 20 mmol) at 0 °C. The free base **11** (1.97 g, 87%) recovered by extraction with CH_2Cl_2 was then dissolved in THF (10 mL) and dropped into LDA in THF (12 mL of a 2.0 M solution, 24 mmol) at −78 °C. The solution was stirred for 1 h at this temperature, after which anhydrous DMF (2.5 mL, 32 mmol) was added dropwise. Water (20 mL) was added when the solution reached room temperature. Organic solvents were evaporated, and the aqueous solution was cooled to 0 °C. $NaBH_4$ (0.95 g, 25 mmol) was added followed by stirring for an additional hour. Extraction with EtOAc and chromatography with EtOAc gave **12** (1.58 g, 63%). ^{1}H NMR: δ 8.64 (s, 1H), 8.36 (d, 1H, J = 5.1 Hz), 7.29 (d, 1H, J = 5.1 Hz), 4.81 (s, 2H). ^{13}C NMR: δ 149.6, 149.2, 143.0, 134.5, 124.3, 60.2.

(4-Azidopyridin-3-yl)methanol (13). A solution of **12** (0.72 g, 5 mmol) and NaN_3 (0.65 g, 10 mmol) in EtOH (5.0 mL) and water (5.0 mL) was refluxed for 7 h and then saturated with NaCl and extracted with EtOAc. Chromatography with EtOAc gave **13** as a white solid (0.37 g, 50%); mp 82−83 °C. ^{1}H NMR: δ 8.52 (s, 1H), 8.49 (d, 1H, J = 5.1 Hz), 7.08 (d, 1H, J = 5.1 Hz), 4.66 (s, 2H). ^{13}C NMR: δ 150.4, 150.3, 146.9, 127.0, 112.7, 59.0. FAB-HRMS calcd for $C_6H_7N_4O(MH^+)$, 151.0620; found, 151.0628. Anal. ($C_6H_6N_4O$) C, H, N.

4-Azidopyridin-3-ylmethyl Chloride (5b). Reaction of compound **13** (0.36 g, 2.4 mmol) with PPh_3Cl_2 (1.28 g, 4.8 mmol) followed by flash chromatography with EtOAc and hexanes (1:1) gave **5b** in 78% yield. ^{1}H NMR: δ 8.56 (s, 1H), 8.55 (d, 1H, J = 5.6 Hz), 7.10 (d, 1H, J = 5.6 Hz), 4.56 (s, 2H). ^{13}C NMR: δ 151.9, 151.1, 147.3, 124.1, 113.0, 38.5.

2-Chloro-5-(4,4-dimethyl-4,5-dihydroxazol-2-yl)pyridine (17). A solution of **16** (10.0 g, 63 mmol) and $SOCl_2$ (9.0 g, 76 mmol) was refluxed for 3 h. Distillation of excess $SOCl_2$ gave 6-chloropyridinyl chloride as a solid residue used directly without purification. A solution of 6-chloropyridinyl chloride (3.5 g, 20 mmol), 2-amino-2-methylpropanol (3.2 g, 20 mmol), and DMAP (100 mg) in CH_2Cl_2 (45 mL) was stirred at room temperature for 2 h and then mixed with ether (100 mL) and filtered. The filtrate was concentrated and stirred with $SOCl_2$ (10 mL) at room temperature overnight. After excess $SOCl_2$ was evaporated, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with aqueous NaOH. The organosoluble fraction was chromatographed to give **17** as a white solid (3.0 g, 71%); mp 105−106 °C. ^{1}H NMR: δ 8.90 (d, 1H, J = 2.6 Hz), 8.17 (dd, 1H, J = 2.6, 6.2 Hz), 7.38 (d, 1H, J = 6.2 Hz), 4.14 (s, 2H), 1.38 (s, 6H). ^{13}C NMR: δ 159.3, 153.9, 149.5, 138.2, 123.9, 123.2, 79.4, 68.0, 28.3. FAB-HRMS calcd for $C_{10}H_{12}ClN_2O(MH^+)$, 211.0638; found, 211.0634.

4-Azido-2-chloro-5-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)pyridine (18). Redistilled *i*-Pr_2NH (2.1 g, 20.8 mmol) in anhydrous THF (30 mL) at −30 °C was treated with *n*-BuLi (11.2 mL of a 1.6 M solution in hexane, 18.0 mmol). Then, the solution was cooled to −78 °C and **17** (2.9 g, 13.8 mmol) in THF (14 mL) was added dropwise. After it was stirred at −78 °C for 5 min, the mixture was cooled to −100 °C and tosyl azide (4.0 g, 20.3 mmol) in THF (7.0 mL) was added. The cooling bath was then removed to allow the reaction mixture to warm to 0 °C. The reaction mixture was poured into saturated NH_4Cl (50 mL) and extracted with EtOAc, followed by chromatography with EtOAc and hexanes (1:2) to give **18** as a solid (2.78 g, 80%); mp 100−102 °C. ^{1}H NMR: δ 8.74 (s, 1H), 7.15 (s, 1H), 4.10 (s, 2H), 1.42 (s, 6H). ^{13}C NMR: δ 157.2, 154.3, 152.3, 149.8, 115.2, 114.0, 78.9, 68.5, 28.3. FAB-HRMS calcd for $C_{10}H_{11}ClN_5O(MH^+)$, 252.0652; found, 252.0662.

4-Azido-6-chloronicotinic Acid (19). A solution of **18** (0.24 g, 0.95 mmol) in EtOAc (10 mL) and *n*-Bu_4NHSO_4 (34 mg) was treated with bleach (5.25% NaOCl, 17 mL) and stirred overnight at room temperature. The residue from extraction with EtOAc and evaporation was dissolved in MeOH (5.0 mL) and mixed with 5% aqueous NaOH (75 mL). After it was stirred overnight, the solution was washed with CH_2Cl_2 and the aqueous phase was acidified with HCl to pH 3 and extracted with CH_2Cl_2. Evaporation gave **19** as a yellow solid (0.10 g, 53%) used without further purification. ^{1}H NMR (CD_3OD): δ 8.74 (s, 1H), 7.44 (s, 1H). ^{13}C NMR (CD_3OD): δ 166.3, 156.0, 153.9, 152.9, 119.9, 116.6. FAB-HRMS calcd for $C_6H_4ClN_4O_2(MH^+)$, 199.0023; found, 199.0024.

(4-Azido-6-chloropyridin-3-yl)methanol (20). Method A. A similar procedure as that for **7** was used by reacting **19** (0.5 g, 2.5 mmol) with ethyl chloroformate (0.28 mL, 2.8 mmol) in the presence of NMM (0.30 mL, 2.8 mmol) and then $NaBH_4$ (0.34 g, 8.9 mmol) followed by chromatography with CH_2Cl_2 and MeOH (10:1) to give **20** (172 mg, 20%). Method B. Compound **19** (100 mg, 0.51 mmol) was refluxed with $SOCl_2$ (2.0 mL) for 2 h, and excess $SOCl_2$ was removed by vacuum. To the residue at 0 °C was added a solution of $NaBH_4$ (67 mg, 1.76 mmol) in water (1.2 mL) at 0 °C followed by stirring for 15 min. The ether extract was chromatographed as above to give **20** (60 mg, 64%). ^{1}H NMR: δ 8.31 (s, 1H), 7.09 (s, 1H), 4.63 (s, 2H). ^{13}C NMR: δ 151.8, 149.8, 149.0, 126.1, 112.9, 58.4. FAB-HRMS calcd for $C_6H_6ClN_4O(MH^+)$, 185.0230; found, 185.0242.

4-Azido-6-chloropyridin-3-ylmethyl Chloride (5d). Compound **20** (80 mg, 0.43 mmol) was converted to **5d** (70 mg, 80%) by PPh_3Cl_2 (280 mg, 0.84 mmol) and NMM (85 mg, 0.84 mmol) and then chromatographed with EtOAc and hexanes (1:1). ^{1}H NMR: δ 8.33 (s, 1H), 7.12 (s, 1H), 4.51 (s, 2H). ^{13}C NMR: δ 152.7, 151.3, 149.5, 123.3, 113.2, 37.8. FAB-HRMS calcd for $C_6H_5Cl_2N_4(MH^+)$, 202.9891; found, 202.9898.

***N*-(5-Azido-6-chloropyridin-3-ylmethyl)ethane-1,2-diamine (21).** A solution of ethylenediamine (0.4 g, 6.7 mmol) and aqueous 50% NaOH (50 μL) in CH_3CN (2.0 mL) at 0 °C was treated dropwise with a solution of **5c** (0.2 g, 1.0 mmol) in CH_3CN (1.0 mL). After 6 h at room temperature, filtration and evaporation gave **21** as a pale yellow oil (0.20 g, 88%) used without further purification. ^{1}H NMR: δ 8.07 (s, 1H), 7.53 (s, 1H), 3.80 (s, 2H), 2.81 (t, 2H, J = 6.2 Hz), 2.65 (t, 2H, J = 6.2 Hz), 1.44 (br s, 3H). ^{13}C NMR: δ 144.6, 140.8, 136.7, 134.4, 127.4, 51.9, 50.0, 41.5. FAB-HRMS calcd for $C_8H_{12}ClN_6(MH^+)$, 227.0812; found, 227.0807.

3-(5-Azido-6-chloropyridin-3-ylmethyl)imidazolidin-2-ylideneamine (1b). A solution of compound **21** (200 mg, 0.88 mmol) in toluene (1.0 mL) was added dropwise into a solution of BrCN (94 mg, 0.88 mmol) in toluene (3.0 mL). The reaction mixture was stirred at room temperature overnight. The solid residue from filtration was washed twice with toluene and dried, giving **1b** as a salt (262 mg, 90%). The free base was obtained by neutralizing in MeOH with K_2CO_3 followed by chromatography with CH_2Cl_2 and 2.0 M NH_3 in MeOH (10:1). ^{1}H NMR: δ 8.09 (d, 1H, J = 1.5 Hz), 7.59 (d, 1H, J = 1.5 Hz), 4.63 (s, 2H), 3.66−3.44 (m, 4H). ^{13}C NMR: δ 160.1, 144.4, 142.6, 135.3, 131.1, 127.9, 47.5, 45.4, 40.9. FAB-HRMS calcd for $C_9H_{11}ClN_7(MH^+)$, 252.0764; found, 252.0758.

3-(5-Azido-6-chloropyridin-3-ylmethyl)-3*H*-thiazol-2-ylideneamine Hydrochloride (1f). A solution of **5c** (120 mg, 0.59 mmol) and 2-aminothiazole (70 mg, 0.70 mmol) was refluxed in *i*-PrOH (20 mL) for 10 h. The solution was evaporated, and the solid was washed with THF (3 × 5 mL) and redissolved in *i*-PrOH and cooled to 0 °C. Compound **1f** (28 mg, 16%) was obtained as a precipitate. ^{1}H NMR: δ 8.07 (s, 1H), 7.70 (s, 1H), 6.84 (d, 1H, J = 5.1 Hz), 6.17 (d, 1H, J = 5.1 Hz), 3.31 (s, 2H). ^{13}C NMR: δ 167.8, 145.1, 142.4, 136.6, 135.1, 129.3, 128.8, 101.3, 46.8. FAB-HRMS calcd for $C_9H_8ClN_6S(MH^+)$, 267.0222; found, 267.0207.

2,2,2-Trifluoro-*N*-(3*H*-thiazol-2-ylidene)acetamide (22). A solution of trifluoroacetic anhydride (TFAA) (5.8 g, 28 mmol) in CH_2Cl_2 was added dropwise into a solution of 2-aminothiazole (2.0 g, 20 mmol) and pyridine (2.4 g, 30 mmol) in CH_2Cl_2

at −65 °C. The solution was stirred at room temperature for 30 min and then washed with water. The aqueous phase was extracted three times with EtOAc. The organic layers were combined, dried, and evaporated to give a solid (3.8 g, 96%), which was recrystallized from EtOAc; mp 174−175 °C. ^{1}H NMR: δ 7.13 (d, 1H, J = 4.1 Hz), 6.83 (d, 1H, J = 4.1 Hz).

***N*-[3-(Substituted-pyridin-3-ylmethyl)-3*H*-thiazol-2-ylidene]-2,2,2-trifluoroacetamide (23c,d,g). General.** A solution of **22** (1 equiv) in DMF was treated with NaH (1.1 equiv) at 0 °C and stirred until no more hydrogen was produced. A solution of **5a**, **5b**, or **5d** (0.8 equiv) in DMF was then added dropwise. The mixture was stirred at room temperature overnight, and solvent was removed in vacuo. Chromatography of the residue gave **23c**,**d**,**g**, respectively.

***N*-[3-(5-Azidopyridin-3-ylmethyl)-3*H*-thiazol-2-ylidene]-2,2,2-trifluoroacetamide (23c).** Yield, 74%; mp 148−149 °C. ^{1}H NMR: δ 8.41 (br s, 1H), 8.35 (d, 1H, J = 2.6 Hz), 7.55 (br s, 1H), 7.21 (d, 1H, J = 4.6 Hz), 6.94 (d, 1H, J = 4.6 Hz), 5.44 (s, 2H). ^{13}C NMR: δ 169.4, 164.9 (q, J = 35.9 Hz), 145.5, 141.9, 137.8, 131.2, 126.5, 126.2, 117.0 (q, J = 283.0 Hz), 112.0, 49.6. FAB-HRMS calcd for $C_{11}H_8F_3N_6OS(MH^+)$, 329.0432; found, 329.0446.

***N*-[3-(4-Azidopyridin-3-ylmethyl)-3*H*-thiazol-2-ylidene]-2,2,2-trifluoroacetamide (23d).** Yield, 67%; mp 138−139 °C. ^{1}H NMR: δ 8.56 (s, 1H), 8.27 (d, 1H, J = 5.6 Hz), 7.37 (d, 1H, J = 4.6 Hz), 7.01 (d, 1H, J = 5.6 Hz), 6.81 (d, 1H, J = 4.6 Hz), 5.16 (s, 2H). ^{13}C NMR: δ 169.2, 164.7 (q, J = 35.9 Hz), 153.4, 151.6, 147.5, 127.4, 120.6, 117.1 (q, J = 283.0 Hz), 112.8, 110.8, 45.8. FAB-HRMS calcd for $C_{11}H_8F_3N_6OS(MH^+)$, 329.0432; found, 329.0449. Anal. ($C_{11}H_7F_3N_6OS$) C, H, N.

***N*-[3-(4-Azido-6-chloropyridin-3-ylmethyl)-3*H*-thiazol-2-ylidene]-2,2,2-trifluoroacetamide (23g).** Yield, 77%; mp 168−169 °C. ^{1}H NMR: δ 8.52 (s, 1H), 7.39 (d, 1H, J = 4.6 Hz), 7.09 (s, 1H), 6.86 (d, 1H, J = 4.6 Hz), 5.22 (s, 2H). ^{13}C NMR: δ 169.0, 164.4 (q, J = 35.9 Hz), 152.8, 149.9, 127.6, 119.7, 116.9 (q, J = 283.0 Hz), 113.0, 111.0, 45.3. FAB-HRMS calcd for $C_{11}H_7ClF_3N_6OS(MH^+)$, 363.0043; found, 363.0029.

3-(Substituted-pyridin-3-ylmethyl)-3*H*-thiazol-2-ylideneamine (1c,d,g). General. Compound **23** (100 mg, ∼0.3 mmol) was dissolved in a solution of NH_3 in MeOH (10 mL of a 2.0 M solution, 20 mmol) and stirred overnight. Evaporation gave the completely deprotected compound, which was purified by chromatography with CH_2Cl_2 and 2.0 M NH_3 in MeOH (10:1).

3-(5-Azidopyridin-3-ylmethyl)-3*H*-thiazol-2-ylideneamine (1c). ^{1}H NMR: δ 8.34 (br s, 1H), 8.30 (br s, 1H), 7.35 (br s, 1H), 6.39 (d, 1H, J = 4.6 Hz), 5.82 (d, 1H, J = 4.6 Hz), 4.92 (s, 2H). ^{13}C NMR: δ 164.0, 145.1, 140.7, 137.4, 133.7, 126.2, 125.3, 98.6, 46.1. FAB-HRMS calcd for $C_9H_9N_6S(MH^+)$, 233.0609; found, 233.0632.

3-(4-Azidopyridin-3-ylmethyl)-3*H*-thiazol-2-ylideneamine (1d). ^{1}H NMR: δ 8.54 (s, 1H), 8.53 (d, 1H, J = 5.6 Hz), 7.09 (d, 1H, J = 5.6 Hz), 6.47 (d, 1H, J = 5.1 Hz), 5.79 (d, 1H, J = 5.1 Hz), 4.83 (s, 2H). ^{13}C NMR: δ 164.1, 151.7, 150.5, 147.0, 127.0, 122.8, 112.7, 98.0, 42.5. FAB-HRMS calcd for $C_9H_9N_6S(MH^+)$, 233.0609; found, 233.0623.

3-(4-Azido-6-chloropyridin-3-ylmethyl)-3*H*-thiazol-2-ylideneamine (1g). ^{1}H NMR: δ 8.36 (br s, 1H), 7.11 (s, 1H), 6.48 (d, 1H, J = 4.6 Hz), 5.79 (d, 1H, J = 4.6 Hz), 4.78 (s, 2H). ^{13}C NMR: δ 163.8, 152.1, 151.5, 149.2, 126.9, 122.0, 112.9, 98.1, 42.1. FAB-HRMS calcd for $C_9H_8ClN_6S(MH^+)$, 267.0220; found, 267.0219.

1-(Substituted-pyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminoimidazole (2c,d,f,g). General. A solution of 2-nitroimino-4-imidazoline (1 equiv) in DMF was treated with NaH (1.1 equiv) at <5 °C. The mixture was stirred at room temperature until no hydrogen was produced and then cooled again to 0 °C. A solution of **5a**−**d** (0.9 equiv) in DMF was added dropwise. The solution was stirred at room temperature for 4−12 h followed by workup and chromatography with CH_2Cl_2 and MeOH (10:1) to give the desired product.

1-(5-Azidopyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminoimidazole (2c). Yield, 34%. ^{1}H NMR (DMSO-d_6): δ 8.35 (d, 1H, J = 2.6 Hz), 8.33 (d, 1H, J = 1.6 Hz), 7.56 (dd, 1H, J = 1.6, 2.6 Hz), 7.39 (d, 1H, J = 2.6 Hz), 7.07 (d, 1H, J = 2.6 Hz), 5.14 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 145.6, 145.2, 140.6, 136.7, 132.6, 125.8, 116.8, 113.7, 45.3. FAB-HRMS calcd for $C_9H_9N_8O_2(MH^+)$, 261.0848; found, 261.0822. Anal. ($C_9H_8N_8O_2$) C, H, N.

1-(4-Azidopyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminoimidazole (2d). Yield, 23%. ^{1}H NMR (DMSO-d_6): δ 8.53 (d, 1H, J = 5.1 Hz), 8.30 (s, 1H), 7.42 (d, 1H, J = 5.1 Hz), 7.26 (d, 1H, J = 2.6 Hz), 7.06 (d, 1H, J = 2.6 Hz), 5.01 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 150.7, 150.6, 146.7, 145.7, 121.3, 117.2, 114.0, 113.4, 42.2. FAB-HRMS calcd for $C_9H_9N_8O_2(MH^+)$, 261.0848; found, 261.0867. Anal. ($C_9H_8N_8O_2 \cdot H_2O$) C, H; N: calcd, 40.27; found, 39.72.

1-(5-Azido-6-chloropyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminoimidazole (2f). Yield, 41%. ^{1}H NMR: δ 8.17 (d, 1H, J = 2.1 Hz), 7.84 (d, 1H, J = 2.1 Hz), 7.28 (d, 1H, J = 2.6 Hz), 7.06 (d, 1H, J = 2.6 Hz), 5.20 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 145.9, 144.8, 140.6, 134.8, 132.7, 129.4, 117.3, 114.2, 45.3. FAB-HRMS calcd for $C_9H_8ClN_8O_2(MH^+)$, 295.0459; found, 295.0462. Anal. ($C_9H_7ClN_8O_2$) C, H; N: calcd, 38.03; found, 37.37.

1-(4-Azido-6-chloropyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminoimidazole (2g). Yield, 46%. ^{1}H NMR (CD_3OD): δ 8.34 (s, 1H), 7.32 (s, 1H), 7.07 (d, 1H, J = 2.6 Hz), 6.95 (d, 1H, J = 2.6 Hz), 5.05 (s, 2H). ^{13}C NMR (CD_3OD): δ 158.0, 152.2, 152.0, 151.5, 121.7, 117.9, 114.6, 114.2, 43.0. FAB-HRMS calcd for $C_9H_8ClN_8O_2(MH^+)$, 295.0459; found, 295.0453. Anal. ($C_9H_7ClN_8O_2$) C, H; N: calcd, 38.03; found, 37.59.

1-(Substituted-pyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminothiazole (2h,i). General. A solution of 2-nitroiminothiazoline (1.2 equiv), **5c**, or 6-chloropyridin-3-ylmethyl chloride (1 equiv) and K_2CO_3 (2.0 eq) in DMF was stirred at 50−55 °C overnight. DMF was then evaporated, and the residue was chromatographed with CH_2Cl_2 and MeOH (10:1) to give the desired product.

1-(6-Chloropyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminothiazole (2h). Yield, 48%; mp 218−219 °C (reported 219−220 °C [39]). ^{1}H NMR: δ 8.45 (d, 1H, J = 2.1 Hz), 7.92 (d, 1H, J = 4.6 Hz), 7.80 (dd, 1H, J = 2.1, 8.2 Hz), 7.53 (d, 1H, J = 8.2 Hz), 7.40 (d, 1H, J = 4.6 Hz), 5.40 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 167.6, 149.6, 139.5, 130.3, 129.9, 127.0, 124.4, 112.6, 49.1.

1-(5-Azido-6-chloropyridin-3-ylmethyl)-1,3-dihydro-2-nitroiminothiazole (2i). Yield, 77%. ^{1}H NMR: δ 8.23 (br s, 1H), 7.96 (br s, 1H), 7.93 (d, 1H, J = 4.6 Hz), 7.41 (d, 1H, J = 4.6 Hz), 5.43 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 167.6, 144.8, 140.3, 134.3, 131.4, 129.8, 129.4, 112.5, 48.8. FAB-HRMS calcd for $C_9H_7ClN_7O_2S(MH^+)$, 312.0098; found, 312.0070. Anal. ($C_9H_6ClN_8O_2S$) C, H; N: calcd, 31.52; found, 31.05.

1-(5-Azido-6-chloropyridin-3-ylmethyl)-2-nitromethyleneimidazolidine (3b). A solution of compound **21** (266 mg, 1.2 mmol), 1,1-bis(methylthio)-2-nitroethylene (200 mg, 1.2 mmol), and a catalytic amount of *p*-toluenesulfonic acid (10 mg) in CH_3CN (5.0 mL) was stirred at room temperature for 16 h and then evaporated. The residue was purified by preparative TLC with CH_2Cl_2 and MeOH (15:1) to give **3b** (62 mg, 18%). ^{1}H NMR (DMSO-d_6): δ 8.92 (br s, 1H), 8.15 (s, 1H), 7.84 (s, 1H), 6.79 (s, 1H), 4.49 (s, 2H), 3.58 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 158.5, 144.3, 139.7, 134.2, 132.8, 128.8, 95.8, 48.0, 45.1, 42.3. FAB-HRMS calcd for $C_{10}H_{11}ClN_7O_2(MH^+)$, 296.0663; found, 296.0660. Anal. ($C_{10}H_{10}ClN_7O_2$) C, H, N.

Radioligand Binding. The affinity of test compounds for the $\alpha4\beta2$ nAChR of M10 cells was determined by displacement of [^{3}H]nicotine binding.[5] The M10 cell line consists of mouse fibroblasts stably transfected with $\alpha4\beta2$ nAChR under the control of a dexamethasone sensitive promoter.[40] The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, 0.1 mg/mL streptomycin, 2 mM L-glutamine, and 0.5 mg/mL geneticin (Gibco Life Technologies, Grand Island, NY) at 37 °C and 5% CO_2/95% air atmosphere. The $\alpha4\beta2$ nAChR was induced by replacing the above medium with one in which the geneticin was deleted and 1 μM dexamethasone (Sigma) was added. Extracts of M10

cell membranes in lysis buffer (50 mM sodium phosphate pH 7.5 containing 2% Triton X-100, 50 mM NaCl, 5 mM EDTA, 5 mM EGTA, 2 mM phenylmethanesulfonyl fluoride, 5 mM benzamidine, and 5 mM iodoacetamide) were incubated overnight with Immulon 4HBX Removawell (Dynex Technologies, Chantilly, VA), which were precoated with monoclonal antibody 299 (Sigma) against the α4 subunit.[9] Then, the microwells were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-Tween 20) and incubated with 10 nM [^{3}H]nicotine (L-[*N*-methyl-^{3}H]nicotine, 81.5 Ci/mmol, NEN Life Science Products, Boston, MA) in competition with a test compound at 25 °C for 120 min. After three washings with PBS-Tween 20, radioactivity remaining on the microwell was subjected to liquid scintillation counting. Insect receptor preparation from *Drosophila* heads and [^{3}H]IMI binding were performed according to our previous procedure.[41] IC_{50} values (molar concentrations of test compounds for 50% inhibition of specific radioligand binding) were determined by iterative nonlinear least-squares regression using the Sigmaplot program (Jandel Scientific Software, San Rafael, CA). The IC_{50} value was converted to a K_i value by the equation of Cheng and Prusoff,[42] i.e., $K_i = IC_{50}/(1 + [L]/K_D)$, with K_D 3.8 nM for the α4β2 subtype in M10 cells[43] and 2.4 nM for the *Drosophila* receptor.[41]

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Supporting Information Available: HPLC analysis data for candidate probes **1b**, **1c**, **1d**, **1f** and **1g** and elemental analysis data for probes **2c**, **2d**, **2f**, **2g**, **2i** and **3b** and intermediates **13** and **23d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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